Extraction, partial purification and characterization of beta-amylase from Gladiolus klattianus

Mamoudou H. DICKO, Université de Ouagadougou
Marjo S. van LEEUWEN
Riet HILHORST
Alfred S. TRAORE, Université de Ouagadougou
Short communication

Extraction, partial purification and characterization of \( \beta \)-amylase from the bulbs of \( G. \) klattianus

M.H. Dicko \(^{a,*} \), M.J.F. Searle-van Leeuwen \(^{b} \), R. Hilhorst \(^{b} \), A.S. Traore \(^{a} \)

\(^{a}\) Laboratoire de Biochimie, CRSBAN, FAST, Université de Ouagadougou, 03 B.P. 7021 Ouagadougou 03, Burkina Faso

\(^{b}\) Department of Food Technology and Nutritional Sciences, Division of Food Chemistry, Wageningen University, P.O. Box 8129, 6700 EV, Wageningen, The Netherlands

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Abstract

The bulbs of \( Gladiolus \) klattianus are used in Burkina Faso in food processing. Activities of \( \alpha \)-amylase and \( \beta \)-amylase were reported within those bulbs for the first time. The purification of the \( \beta \)-amylase involved buffer extraction, ammonium sulfate precipitation and gel filtration chromatography. The enzyme was purified 47 fold with 75\% yield, giving a final specific activity of 2360 \( \text{U/mg} \). The \( \beta \)-amylase from \( G. \) klattianus was shown to be a heterodimer protein of 60 and 12 kDa subunits. Optimum pH and temperature for the activity were 5.5\( \degree \)C and 55\( \degree \)C, respectively. The abundance of \( \beta \)-amylase in \( G. \) klattianus suggests its possible application for biotechnological purposes.

Keywords: \( \alpha \)-amylase; \( \beta \)-amylase; Starch; Bulb; \( Gladiolus \) klattianus

1. Introduction

\( \beta \)-amylase (\( \alpha \)-1,4-glucan maltohydrolase) [E.C: 3.2.1.2] is a maltogenic amylase widely distributed among higher plants. In the last decades, enzyme separation and purification has become increasingly important because of the evolving application of, notably, barley and soya \( \beta \)-amylases in industry (Somers et al., 1989). The practical interest of \( \beta \)-amylase centres on its capacity to produce maltose syrups from starch (Boivin, 1997). The need for new plant sources of \( \beta \)-amylase still remains (Rashad et al., 1995), for formulation of enzymatic complexes of starch degrading enzymes for biotechnological applications.

Ethnobotanical investigations conducted by our research group have found that the aqueous extracts of the bulbs of \( Gladiolus \) klattianus and the leaves of \( Boscia \) senegalensis (Dicko et al., 1996a,b) are empirically used in Burkina Faso for the preparation of cereal based diets such as ‘Zoom-koom’ and ‘dégue’. The mixture of aqueous extracts with cereal flour allows production of sweet products with improved taste. The purpose of this study was to isolate the major starch-degrading enzyme from a plant of local importance, \( G. \) klattianus, and to study some of its properties.

2. Methods

2.1. Enzyme purification

Unless otherwise indicated, all enzyme purification steps were carried out at 4\( \degree \)C, and all buffers contained 0.02\% (w/v) sodium azide to prevent microbial growth.

Ungerminated fresh bulbs (100 g) of \( G. \) klattianus Hutch (Iridaceae) were peeled, cut and then ground in an ice-cold porcelain mortar with 200 ml of 50 mM sodium maleate, pH 6, containing 3 mM CaCl\(_2\), 20 mM cysteine, 0.1\% (w/v) NaCl and 2\% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 10 000 g for 20 min at 4\( \degree \)C and the supernatant was fractionated by ammonium sulfate precipitation. Most enzyme activity was associated with the fraction precipitating between 40\% and 65\% saturated ammonium sulfate. After dialysis, the fraction was lyophilized and kept at -20\( \degree \)C prior to further purification. An aliquot of the lyophilized fraction was dissolved in 100 mM sodium acetate buffer, pH 5, containing 150 mM NaCl and 3 mM
dithiothreitol. The fraction was applied twice to gel filtration column chromatography (Superdex 75 pg, 60 × 16 mm, FPLC®, Pharmacia) equilibrated in the same buffer. The protein elution (monitored at 254 nm), was performed at a flow rate of 0.8 ml/min with the same buffer. Fractions containing amylase activity were pooled. The native molecular mass of the enzyme was determined with the same gel filtration column calibrated with low molecular mass protein standards (Amersham Pharmacia Biotech calibration kit).

2.2. Enzyme assays

β-Amylase activity was determined measuring the release of reducing sugars with the disodium 2,2'-bicinchoninate assay (Garcia et al., 1993) as previously modified (Dicko et al., 1999). Total protein was quantified by the method of Sedmak and Grossberg (1977). The β-amylase activity was also determined using p-nitrophenyl maltopentaoside as a specific substrate (McCleary and Codd, 1989). The azurine-crosslinked-amylose was used to determine α-amylase activity (McCleary and Sheehan, 1989).

2.3. Gel electrophoresis

The degree of purification and the molecular mass of the enzyme were determined by SDS-PAGE on a 10–15% polyacrylamide gel under reducing conditions with the PhastSystem unit (Amersham Pharmacia Biotech). The gel was Coomassie Brilliant Blue stained. Low-molecular mass standard proteins (14.4–94 kDa) were used as calibration.

2.4. Enzymic properties

The optimum activity and the stability of the enzyme as a function of pH and temperature were determined as previously described (Dicko et al., 1999).

Changes of optical rotation of sugars formed after the enzyme reaction were determined as a function of time as previously described by Kohno et al. (1989), using a Ceti Polaris polarimeter (Belgium).

The analysis of released sugars after incubation of the enzyme with starch was carried out by high performance anion exchange chromatography as described by Mutter et al. (1998). Maltodextrins (Spreda, Burghof, Switzerland) were used as standard glucose oligomers.

3. Results and discussion

3.1. Enzyme purification

Since bulbs of *G. klattianus* Hutch (*Iridaceae*) are traditionally used in preparation of cereal based diets, they were expected to contain a variety of glycolytic enzymes. Using the appropriate substrates, it was shown that the crude extract contained both α-amylose and β-amylose. β-amylose appears to be the major starch degrading enzyme of the bulbs and its activity in the crude extract was approximately 440 U/g of fresh matter; that value is similar to the activity of β-amylose from barley malt (400–500 U/g of fresh matter; Boehringer, Mannheim, Germany). The precipitation by ammonium sulfate and two consecutive elutions of the fraction on FPLC gel filtration column (Superdex 75), allowed purification of the β-amylose 47 fold with a yield of 75% from the crude extract (Table 1). The final specific activity was 2360 U/mg. The yield of the purification is relatively high and comparable to the high yield obtained by Chang et al. (1996) for the purification of the β-amyloses from sweet potatoes (73–76% yield). Analysis of the enzyme by SDS-PAGE (under denaturing conditions) showed two protein bands of, respectively 12 and 60 kDa. However, a single symmetrical peak of about 72 kDa, with β-amylose activity, was found by gel filtration chromatography. These results suggest that the enzyme is a dimeric protein constituted of two protein subunits of 12 and 60 kDa, with an estimated total molecular mass of 72 kDa. These results need to be confirmed by the isoelectric focusing and native-PAGE analysis of the enzyme. This molecular mass (72 kDa) is higher than that of monomeric β-amyloses from pea epicotyl (57 kDa) (Lizotte et al., 1990), ginseng (63 kDa) (Yamasaki et al., 1989), and alfalfa (61 kDa) (Kohno et al., 1989). Several multimeric β-amyloses have been reported (Saha et al., 1987; Chang et al., 1996) but most of them are composed of homomeric subunits. The enzyme isolated in this work is free of many contaminant proteins that could interfere with the analysis of its catalytic properties.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U/mg)</th>
<th>Purification fold</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>875.7</td>
<td>43 960</td>
<td>50.2</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitation</td>
<td>98.1</td>
<td>42 379</td>
<td>432</td>
<td>8.6</td>
<td>96</td>
</tr>
<tr>
<td>Superdex 75 (1)</td>
<td>18.4</td>
<td>33 377</td>
<td>1814</td>
<td>36</td>
<td>76</td>
</tr>
<tr>
<td>Superdex 75 (2)</td>
<td>14.0</td>
<td>33 040</td>
<td>2360</td>
<td>47</td>
<td>75</td>
</tr>
</tbody>
</table>
3.2. **Optimum parameters of activity and stability**

The enzyme exhibited optimum activity for hydrolysis of starch at pH 5.5. It was stable in the pH range of 4.5–8 and the activity dropped gradually at more alkaline or acidic values. This behavior is similar to most plant β-amylases. The enzyme displayed optimum activity at 55°C. The enzyme was fully stable up to 50°C for 1.5 h incubation in the absence of the substrate, and 80% of the original activity was retained after heat treatment at 55°C for 1.5 h.

3.3. **Action pattern**

Analysis of the action pattern of the enzyme by high performance anion exchange chromatography, showed that it produced only maltose after 5 min incubation with potato starch. This indicates the exo-fashion mode of hydrolysis of the enzyme. Furthermore, based on the changes of optical rotation of the released maltose (Yamasaki et al., 1989; Kohno et al., 1989), it was identified as β-anomer. These results show that the enzyme is a β-amylase.

4. **Conclusion**

A β-amylase could be purified from the inexpensive raw plant material, bulbs of *G. klattianus*. The recovery of the enzyme activity (75%) suggests its possible large-scale production by either techniques used above or other separations such as ion-exchange chromatography. The high activity of β-amylase within these bulbs justifies their empirical use in Burkina Faso for food processing, by enzymatic conversion of starch. Due to the economical importance of β-amylase in high maltose-containing syrup production and in brewing (Norris and Lewis, 1985), *G. klattianus* could be a potential candidate as a novel source of β-amylase. The wide distribution of the plant makes it available in almost all West Africa.

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**References**


